“STUDY OF ENVIRONMENTAL CARCINOGENS’ INTERFERENCE IN BIO-MOLECULAR KINETICS OF CELL CYCLE REGULATORY MACHINERY USING SYSTEMS BIOLOGY APPROACH AND THEIR PROTECTION BY NANO-PARTICLES”

SYNOPSIS SUBMITTED

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Background

According to National Institute of Environmental Health Sciences (NIEH), there are more than 100 different types of cancers. As per the report submitted by the scientists of NIEH and NTP, there are around 200-300 carcinogens that include environmental carcinogens like pesticides, polycyclic aromatic hydrocarbons, cigarette smoke, tobacco etc (National Toxicology Program). The Environmental Protection Agency (EPA) has reported that the diesel exhaust contains fine particles that pose a serious threat to human health. These fine particles from the diesel exhaust can pass through the nose and the throat and damage the lungs. These particles contribute in worsening the respiratory conditions and developing diseases like Asthma and Bronchitis.

Exposure of certain substances and chemicals that can cause cancer are called carcinogens. There are some carcinogens that act directly on DNA and cause DNA damage, while there are some which affect the cell cycle regulation by binding to cell cycle regulatory proteins leading to cancer in different ways. For example, they may cause cells to divide at a faster than normal rate, which could increase the chances that changes in DNA will occur and the mutated DNA will pass on to the daughter cells.

Increased air pollution, containing carcinogenic particulate matter smaller than 2.5 μm ($PM_{2.5}$), has gained particular attention in recent years as a causative factor in the increased incidence of respiratory diseases, including lung cancer. Makoto Enomoto and his colleagues in 2008 conducted extensive studies on carcinogenicity using numerous chemical compounds and indicated the importance of dosage and duration of exposure of carcinogen to evaluate the carcinogenicity and other toxicity of that chemical agent. Cell cycle regulation is a very complex process with a large number of proteins involved in this. At each phase of the cell cycle, there are

checkpoints to check if the cell moving to next phase is in appropriate condition and healthy state for further division. If the cell is very small in size, it is stopped at the very first checkpoint. In S phase, the DNA is duplicated and then checked for any mutations, or mismatches. If it is found that there are discrepancies, the S/G2 checkpoint, stops the cell from moving into the next phase and the DNA repair mechanism starts. The DNA repair mechanisms are important as the cells can become cancerous by a slight deregulation in these repair pathways. There are various CDK-Cyclin complexes that regulate and govern the transition of the cell from one phase to next phase.

There are at least two types of cell cycle regulatory mechanisms that have been recognized. The first mechanism contains a cascade of protein phosphorylations that allows a cell to move from one stage to the next which involves a highly regulated kinase family. The kinases are activated when they are in association with their cyclin subunits. Each CDK-Cyclin complex has its unique substrate specificity. Another mechanism has a set of checkpoints that checks whether the critical events of the cell cycle are complete or not. If the critical events have not been completed, the cell gets restricted and the progression is delayed. The transition between the cell cycle stages is well programmed because of the finely regulated activity of phosphorylation and dephosphorylation of the CDK-cyclin complexes. (Collins K. et al. 1997)

Cancer is a multi-step process, involving mutations in a number of genes responsible for cell cycle regulation including tumor suppressor and proto oncogenes along with mutations in genes producing cell to cell adhesion proteins, in case of malignant tumors. There is now growing recognition that most of the human cancers are due to exposure to Xenobiotics (carcinogens) in the environment and that they are hence ultimately preventable (Verma N. et al. 2012; Boffetta et

al. 1997; Melendez-Colon et Al. 1999). Also, it is believed that these xenobiotics may have a possible role in activating oncogenic viruses (Environmental Determinants of Human Cancer). PAHs constitute a considerable group of these carcinogens. Human exposure to PAHs may occur from a number of sources and is almost inevitable. Many of them cause carcinogenesis by reacting/damaging the DNA and/or a number of proteins.

Brandon D Jeffy et al. (2000) has reported that BaP and its derivative BDPE affects the cell cycle kinetics in MCF-7 Breast cancer cells. When these cells were treated with BaP, they were arrested in S phase and showed increased levels of p53 and the cellular content of p21 remained unaltered. There are evidences that tobacco smoke induces expression of cyclin D1 and PCNA and the AP-1 target genes involved in promoting cell cycle progression (Zhong et al. 2005).

Recently some of the nanoparticles have been employed in scavenging the high molecular weight poly cyclic aromatic hydrocarbons (PAHs) from the contaminated soils (Karnchanasest and Santisukkasaem, 2007). Amphiphilic polymer nanoparticles have been used as nano-absorbent for pollutants in aqueous phase (Noh et al. 2008). In a recent report, Bochra et al. (2011), have shown the potential application of titanate nanotubes as solid-phase extraction adsorbents for seven PAHs: acenaphtylene, acenaphthene, anthracene, fluorene, phenanthrene, fluoranthene and pyrene, from the environmental water samples, where the recoveries of PAHs ranged from 90% to 100%.

The scavenging capacities of the nanoparticles for PAH and other pollutants could probably be attributed to their higher affinity towards the xenobiotics (Karnchanasest and Santisukkasaem, 2007). The structural properties and surface chemistry of nanoparticles are the players, further, extremely high surface area to volume ratio results in multiple enhancements of such properties.

As UF-TiO2 has been shown to scavenge the PAHs efficiently from the soil and water sample from the environment (Karnchanasest and Santisukkasaem, 2007), UF-TiO_2 has also shown its scavenging ability for PAHs in the biological/cellular system, preventing the interactions of PAHs with the biomolecules. (Dhasmana A et al. 2014). This will not only help prevent the initiation and development of Cancer only, but a number of pathological conditions caused by exposure to PAHs, some initial *in vitro* experiments have been performed with UF-TiO2 and BaP, and it has been found that co-exposure of the A549 cells to the two, results in antagonistic toxicity of both the agents (Dhasmana A et al. 2014 and 2016).

Till last two decades, the treatment of cancer was mainly focused on the exploitation of the molecular biology, but now scientists are more focused on finding the critical oncogenes or the tumor suppressor genes that are responsible for the development of cancer. The upcoming field of computational biology, for the treatment of any disease including cancer, works on creating the mathematical models for the simulation of the dynamic biological processes. This will help in the development of the targeted treatment. With the ultimate goal of understanding the response of the cancer populations, an increasing no.of mathematical, physical, engineering and computational techniques are being applied to various aspects of tumor growth. (Enderling H. et al. 2014). For the optimization of the patient care, *in silico* trials that predict patient specific response to various dose schedules or treatment combinations are becoming invaluable tools. (Enderling H. et al. 2014; Fu F. et al. 2015).

The recent trend in cancer research is based on the utilization of computational approaches that include new mathematical and computational algorithms. This new approach clearly shows the dynamic interplay that exists between experimental biology and other quantitative sciences. (Carro, MS et al. 2010) This approach of cancer systems biology can be applied at different

strata ranging from an individual cell to an entire tissue, a patient with the primary location of the tumour and possible locations of metastases, or even to any combinations of these situations.(Huang, SS et al. 2013; Pascal, J et al. 2013);

With the help of these system - network biology approach, we aim at designing the cell cycle regulatory pathway. A dynamic model of cell cycle regulation will be prepared along with the initial concentrations of cell cycle regulatory proteins. Inhibitors or carcinogens like PAHs and TSNAs will also be incorporated in the cell cycle regulation kinetic model. With this we aim at finding the affect of the carcinogens on different cell cycle regulatory proteins and how this carcinogen- protein interaction will affect the cell cycle regulatory pathway and other pathways, thus creating a network of networks. We will analyze how the change in one cell cycle regulatory protein will affect the functioning of other pathways.

The study will help to determine the adverse effects of environmental carcinogens and their metabolites on the cell regulatory pathway proteins and help in formulating nanoparticles for the therapeutic use in order to reduce the risk of development of cancer and/or other manifestations in the PAH and other Xenobiotics exposed populations. The potent carcinogens under study will be PAHs and TSNAs.

Objectives

1) Identification of most probable biomolecular targets of environmental carcinogens (BaP & NNK) among the cell cycle regulatory proteome once they enter the biological system.

2) Designing of cell cycle regulatory biomodel along with its kinetics and determining the impact of environmental carcinogens on normal cell cycle regulation.

3) Construction of network system for functional coverage of most potent biomolecular target of environmental carcinogens among the cell cycle regulatory proteome.

4) Determination of the binding efficiencies of environmental carcinogens with carbon based nanoparticles, environmental carcinogens adsorption load over nanoparticles and comparison of binding efficiencies of carcinogens with their biomolecular targets and nanoparticles.

Hypothesis

In this study we will try to find out the possible cell cycle regulatory protein targets for BaP and NNK. We will try to find how these carcinogens will bind to the target proteins and how they will cause the change in the functionality of the proteins causing its impact on the cell cycle regulation as well its impact on the other systems related to that protein. When the carcinogen will bind with its target protein, it will change the kinetics of the reactions carried out further. This change in kinetics can change the whole functioning of the cell or this change can bring out further changes in the pathways related to that protein or that particular reaction.

In this study we hypothesized that the environmental carcinogens will bind with the cell cycle regulatory proteins which in turn can trigger the cell making it cancerous. This binding can modulate the metabolic processes of the cell by surpassing some important checkpoints of the cell cycle regulation which leads to tumor formation.

The carcinogen can also bind with the DNA repair pathway enzymes and proteins. This will hamper the normal functioning of the DNA repair pathway which can lead to the development of certain mutations. The DNA repair pathway is a very critical and important pathway. This recognizes the harmful mutations, mismatches or deletions in the DNA and corrects them before the cell is allowed to move into the next phase of the cell cycle. When this pathway gets hampered, the cell moves to the next phase without proper checking of the DNA.

There is a very complex machinery of proteins which regulates the cell cycle. Change in the functioning of any single protein can lead to huge changes in the whole biological system. This

study aims at finding the impact of these functional changes on the whole system by considering some important cell cycle regulatory proteins.

The nanoparticles have tendency to scavenge the high electron rich species which already proofed in environmental system for the decontamination of soil and water by removal of Polycycle Aromatic Hydrocarbon, by following of these studies we hypothesized that NPs can also play protective role in biological systems by attenuating the toxic effects of environmental carcinogens. These NPs may be having preferential binding towards the carcinogens and make these carcinogens unavailable to react with the cellular system inside or outside of cell vicinity. This hypothesis will help in finding how much environmental carcinogens will be adsorbed by the nanoparticles over its surface. This can lead to finding the safe dose of the nanoparticles for the scavenging activity against the carcinogens in the biological systems.

Methodology

1) Identification of most probable biomolecular targets of environmental carcinogens (BaP & NNK) among the cell cycle regulatory proteome once they enter the biological system.

The Procurement of 3D structures of cell cycle regulatory proteome will be done by the RCSB Protein Data Bank and if those 3D structure of cell cycle regulatory proteins will not be available in RCSB database then the structures will be generated by the use of Homology protein modeling and Multi template threading technique. Carcinogens' and their metabolites' *.mol* or .pdb files will be downloaded from chemical public databases and further, molecular interactions of proteins with carcinogens will be analyzed using molecular docking tool like Autodock 4.2. The biomolecules/proteins exhibiting good binding efficiencies with carcinogens will be considered as its probable target.

2) Designing of cell cycle regulatory biomodel along with its kinetics and determining the impact of environmental carcinogens on normal cell cycle regulation.

Cell cycle regulation biomodel will be prepared by using BioModel, Cell Designer and Cytoescape etc. The components of cell cycle regulatory proteomes will be identified by using KEGG, BRENDA and available research literatures. The initial concentrations and reaction kinetic equations will be incorporated in every node and edge of reactions. Initial concentrations will be found by the help of the information available through the public domain databases like BioModel and intense review of literature will be done to attain the initial molecular concentrations close to that found in the biological systems. The initial concentrations and biomolecular kinetics of the carcinogens and other inhibitors will also be constructed by the

same procedure as used for the remaining reactions. Impact of the carcinogens in the biomodel kinetics will be observed by differences in the reaction kinetics of normal cell cycle and abnormal cell cycle (abnormality induced by the carcinogens).

Further the protein-protein interaction will be used to functional loss of most probable biomolecular targets. The active site characterization and binding analysis would be analyzed using Zdock, Hex and PatchDock programmes. Discovery studio visulizer and PyMol will be used for the visualization tool to look insight at atomic level for the complete biomolecular interaction between receptors and ligands. Furthermore, The complex (containing Environmental Carcinogens + target biomolecular protein) will be used for the docking analysis. After that comparing the results of docking of protein-protein (Protein + Functional protein partner) and protein-complex (Protein-carciongen + Functional protein partner), this result will help to conclude the impact of environmental carcinogens on its target biomolecule functioning.

3) Construction of network system for functional coverage of most potent biomolecular target of environmental carcinogens among the cell cycle regulatory proteome.

For the system network and functional analysis, the most probable biomolecular target will be indentified from the above objective which will deregulate the most of events in the cell cycle pathway, then network motif and system networking methods will be used to identify the ultimate impact of that hampered biomolecule/s on the whole system network those may get regulated by that target biomolecule. Different gene- protein networking databases will be used for this analysis.

4) Determination of the binding efficiencies of environmental carcinogens with carbon based nanoparticles, environmental carcinogens adsorption load over nanoparticles and

comparison of binding efficiencies of carcinogens with their biomolecular targets and nanoparticles.

Nanotube Modeler will be used for the modeling of nanoparticles (Carbon Nanotube and Fullerene). Material studio will be the most suitable tool to identify the adsorption load/ loading capacity of the pollutant/carcinogens over the nanoparticle surface. Remaining AutoDock will be use for the comparison of binding affinity of carcinogens with nanoparticles and carcinogens with biomolecules.

Major Findings

a. This proposed study will provide in-depth knowledge of intrinsic molecular mechanism of carcinogens induced toxicity/transformation.

b. System biology and network analysis approaches will help in attaining the in-depth understanding of modulations in proteins network coverage and kinetics of cell cycle regulatory proteins.

c. We will construct the whole kinetics biomodel of cell cycle regulatory proteins with actual initial concentration of each and every involved reaction species, with kinetic rate of reactions and cellular compartments where these biochemical reactions will be done. This kind of study will be very helpful and less painful for the other researchers and scientist who has interest in analysis of cell cycle regulation proteins.

d. This proposed study will enlighten the fate of the nanoparticles in the biological system and also disclose the hidden scavenging potential of low dose carbon based nanoparticles to attenuate the toxicity induced by cigarette smoke, vehicle and industrial exhaust carcinogens and can also be applied in drug over dose toxicity and chemical war situation.

Significance

a. This proposed study will reveal and pinpoint the hidden components of cell cycle regulatory machinery related proteins which will be responsible for the deregulation of the whole kinetics in the presence of environmental carcinogens. The resultant of this study will dig out the in-depth evidences of intrinsic biomolecular mechanism of carcinogen induced transformation.

b. Systems network analysis approaches will help in attaining the exhaustive understanding of modulations in proteins network coverage and kinetics of cell cycle regulatory proteins. By the help of Systems biology approach, we aim at finding how the modulation of a single protein by environmental carcinogens will affect the whole cell cycle regulation. This will help in future researches in development of personalized cancer treatment. This study will also provide a large amount of information from different literatures and the kinetics of cell cycle regulatory proteins.

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